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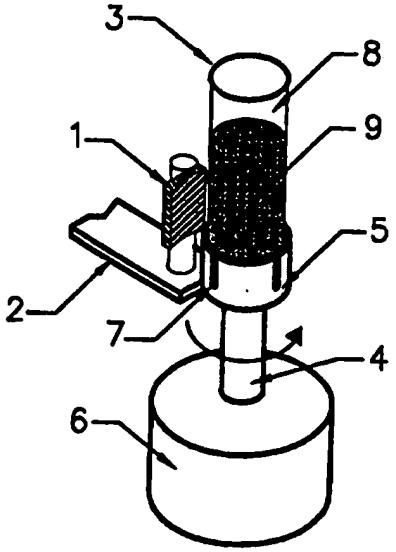
(54) Title: APPARATUS AND METHOD FOR MIXING AND SEPARATION EMPLOYING MAGNETIC PARTICLES

(57) Abstract

An apparatus and method for carrying out the affinity separation of a target substance from a test medium operates by mixing magnetic particles having surface immobilized ligand or receptor with the test medium to promote affinity binding reaction between the ligand and the target substance. The test medium with the magnetic particles in a suitable container is removably mounted in an apparatus of the present invention and magnetic field gradient is created in the test medium. This gradient induces the magnetic particles to move relative to the test medium, thereby effecting mixing. Depending on the embodiment employed, the mixing is achieved either by the movement of an electromagnet or a permanent magnet relative to a stationary container or the movement of the container relative to a stationary magnetic source. After sufficient time for the affinity reaction to occur, the movement of the magnetic gradient is ended, whereby magnetic particles are immobilized on the inside wall of the container nearest to the magnetic source. The remaining test medium is removed while the magnetic particles are retained on the walls of the container and the test medium or the particles subjected to further processing.



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APPARATUS AND METHOD FOR MIXING AND SEPARATION EMPLOYING MAGNETIC PARTICLES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to apparatus and methods for mixing and separation of magnetic particles for the purpose of isolating substances of interest from a nonmagnetic liquid test medium.

2. Description of Related Art

Magnetic separation of biomolecules and cells based on magnetic particles and employing biospecific affinity reactions is advantageous in terms of selectivity, simplicity, and speed. The technique has proved to be quite useful in analytical and preparative biotechnology and is now being increasingly used for bioassays and isolation of target substances such as cells, proteins, nucleic acid sequences and the like.

As used herein, the term "receptor" refers to any substance or group of substances having biospecific binding affinity for a given ligand, to the substantial exclusion of other substances. Among the receptors susceptible to biospecific binding affinity reactions are antibodies (both monoclonal and polyclonol), antibody fragments, enzymes, nucleic acids, lectins and the like. The term "ligand" refers to substances such as antigens, haptens, and various cell associated structures having at least one characteristic determinant or epitope, which substances are capable of being biospecifically recognized by and bound to a receptor. The term "target substance" refers to either member of a biospecific binding affinity pair, i.e., a pair of substances or a substance and a structure exhibiting a mutual affinity of interaction, and includes such things as biological cells or cell components, biospecific ligands, and receptors.

Affinity separation refers to known process techniques where a target substance mixed with other substances in a liquid medium is bound to the surface of a solid phase by a biospecific affinity binding reaction. Substances, which lack the specific molecule or structure of the target substance, are not bound to the solid phase and can be removed to effect the separation of the bound substance or vice versa. Small particles, particularly polymeric spherical particles as solid phase, have proved to be quite useful, as they can be conveniently coated with biomolecules, provide a very high surface area, and give reasonable reaction kinetics. Separations of the particles containing bound target substance (bound material) from the liquid medium (free material) may be accomplished by filtration or gravitational effects, e.g., settling, or by centrifugation.

Separation of bound/free fractions is greatly simplified by employing magnetizable particles which allows the particle bound substance to be separated by applying a magnetic field. Small magnetizable particles are well known in the art as is their use in the separations involving immunological and other biospecific affinity reactions. Small magnetizable particles generally fall into two broad categories. The first category includes particles that are permanently magnetized, and the second comprises particles that become magnetic only when subjected to a magnetic field. The latter are referred to as paramagnetic or superparamagnetic particles and are usually preferred over the permanently magnetized particles.

For many applications, the surface of paramagnetic particles is coated with a suitable ligand or receptor, such as antibodies, lectins, oligonucleotides, or other bioreactive molecules, which can selectively bind a target substance in a mixture with other substances. Examples of small magnetic particles or beads are disclosed in U.S. Patent No. 4,230,685, issued October 28, 1980; U.S. Patent No. 4,554,088 issued November 19, 1985; and U.S. Patent No. 4,628,037, issued December 9, 1986. The use of paramagnetic particles is taught in publications, "*Application of Magnetic Beads in Bioassays*," by B., Haukanes, and C. Kvam, *Bio/Technology*, 11:60-63 (1993); "*Removal of Neuroblastoma Cells from Bone Marrow with Monoclonal Antibodies Conjugated to Magnetic Microspheres*" by J.G. Treleaven *et.al.*,

Lancet, January 14, 1984, pages 70-73; *"Depletion of T Lymphocytes from Human Bone Marrow,"* by F. Vartdal *et.al.*, *Transplantation*, 43: 366-71 (1987); *"Magnetic Monosized Polymer Particles for Fast and Specific Fractionation of Human Mononuclear Cells,"* by T. Lea *et.al.*, *Scandinavian Journal of Immunology*, 22: 207-16 (1985); and *"Advances in Biomagnetic Separations,"* (1994), M. Uhlen *et.al.* eds. Eaton Publishing Co., Natick, MA.

The magnetic separation process typically involves mixing the sample with paramagnetic particles in a liquid medium to bind the target substance by affinity reaction, and then separating the bound particle/target complex from the sample medium by applying a magnetic field. All magnetic particles except those particle that are colloidal, settle in time. The liquid medium, therefore, must be agitated to some degree to keep the particles suspended for a sufficient period of time to allow the bioaffinity binding reaction to occur. Examples of known agitation methods include shaking, swirling, rocking, rotation, or similar manipulations of a partially filled container. In some cases the affinity bond between the target substance and the paramagnetic particles is relatively weak so as to be disrupted by strong turbulence in the liquid medium. In other cases biological target substances such as cells, cellular fractions, and enzyme complexes are extremely fragile and will likewise be disrupted or denatured by excess turbulence.

Excess turbulence is just one of several significant drawbacks and deficiencies of apparatus and methods used in the prior art for biomagnetic separations. The specific configuration of a magnetic separation apparatus used for separating particle-bound target complex from the liquid medium will depend on the nature and size of magnetic particles. Paramagnetic particles in the size range of 0.1 to 10 μm are readily removed by means of commercially-available magnetic separation devices. Examples of such magnetic separation devices are the Dynal MPC series of separators manufactured by Dynal, Inc., Lake Success, NY; and BioMag Separator series devices manufactured by PerSeptive Diagnostics, Cambridge, MA; and a magnetic separator rack described in U.S. Patent No. 4, 895,650. These devices employ permanent magnets located externally to a container holding a test medium and provide only for separation. Mixing of the paramagnetic particles in the test medium

for affinity binding reaction must be done separately. For example, Dynal MPC series of separators requires a separate mixing apparatus, a Dynal Sample Mixer, for agitating the test media. The process must be actively monitored through various stages of mixing, washing, and separation, and requires significant intervention from the operator. Accordingly, the efficiency of these devices is necessarily limited by the skill and effectiveness of the operator.

U.S. Patent No. 4,910,148, issued March 20, 1990, describes a device and method for separating cancer cells from healthy cells. Immunoreactive paramagnetic particles and bone marrow cells are mixed by agitating the liquid medium on a rocking platform. Once the particles have bound to the cancer cells, they are separated from the liquid medium by magnets located externally on the platform. Although such mixing minimizes the liquid turbulence, it does not provide an efficient degree of contact between the particles and the target substance. Moreover, the utility of this device is limited to the separation of cells from relatively large sample volumes.

U.S. Patent No. 5,238,812, issued August 24 1993, describes a complicated device for rapid mixing to enhance bioaffinity binding reactions employing a U-tube-like structure as mixer. The U-tube is rapidly rocked or rotated for 5 to 15 seconds to mix the magnetic particles in the test medium, and then a magnet is brought in close proximity to the bottom of the U-tube to separate the magnetic particles. As stated in the '812 patent, its utility is limited to treating very small volumes ($< 1000\ \mu l$) of test medium.

U.S. Patent No. 5,336,760, issued August 9, 1994, describes a mixing and magnetic separation device comprising a chamber attached to a platform with one or more magnets located close to the container and an intricate mechanism of gears and motor to rotate the platform. Immuno-reactive paramagnetic particles are mixed in the test medium by first placing a stainless steel "keeper" between the chamber and the magnet to shield it from the magnetic field, and then rotating the platform between vertical and horizontal positions. The particles in the test medium are mixed by end-over-end movement of the chamber to facilitate binding of the target substance. Following the mixing, the "keeper" is removed so that the magnetic particles are captured by the exposed magnetic field. Although the '760 patent provides

the convenience of a single apparatus for mixing and separation, it requires a complicated mechanism. Moreover, agitation of the liquid medium by end-over-end rotation does not mix relatively buoyant particles efficiently, and the liquid turbulence may also shear off or damage the target substance.

U.S. Patent No. 5,110,624, issued May 5, 1992, relates to a method of preparing magnetizable porous particles and describes a flow-through magnetically stabilized fluidized bed (MSFB) column to isolate proteins from cell lysate. The MSFB column is loosely packed with a bed of magnetizable particles and equipped with means of creating a stationary magnetic field that runs parallel to the flow of solution through the column. The particles are maintained in a magnetically stabilized fluidized bed by adjusting the rate of flow of the solution and the strength of the magnetic field. As stated in the '624 patent, the MSFB simply increases the void volume of the bed to prevent the fouling of the particle bed or plugging of the flow. Although MSFB can provide superior mixing, it is a complicated technique requiring precise adjustment of the flow rate and magnetic strength so that the combined effect of fluid velocity and magnetic attraction exactly counterbalances the effect of gravity on the particles. The design of MSFB is, however, not optimized for use with small test volumes, and cannot be made optimal for applications such as bioassays or cell separations; therefore, its utility is somewhat limited.

The major drawbacks of the disclosed devices and methods are apparent from the foregoing review of the prior art. Although existing devices and methods afford certain advantages in performing magnetic separation, the known procedures have limitations, including the requirement for a separate or mechanically complex mixing mechanism, as well as various process constraints and inefficiencies. Thus, it is desirable to provide devices for magnetic separation which are of relatively simple construction and operation, which can be adapted to process large or small volumes of test liquid, and which can process multiple test samples simultaneously. Additionally, it is desirable to provide mixing and separation processing on a single device which maximizes the mixing efficiency of the paramagnetic particles in the test medium without causing significant liquid turbulence.

SUMMARY OF THE INVENTION

According to the present invention, the affinity separation of a target substance from a test medium is carried out by mixing magnetic particles bearing surface immobilized ligands or receptors to promote specific affinity binding reaction between the magnetic particles and the target substance. The test medium with the magnetic particles in a suitable container is removably mounted in an apparatus of the present invention and a magnetic field gradient is created in the test medium. This gradient induces the magnetic particles to move relative to the motionless test medium. Depending on the embodiment of the invention employed, the movement of the magnetic source or of the container is started to mix the magnetic particles in the test medium and is continued for a sufficient time to ensure optimum binding of the target substance by affinity reaction. The movement of the magnet or the container is then stopped, whereby magnetic particles are immobilized on the inside wall of the container nearest to the magnet. The remaining test medium may be removed while the magnetic particles are retained on the walls of the container and subjected to further processing, as desired.

BRIEF DESCRIPTION OF THE DRAWINGS

The objects and features of the present invention, which are believed to be novel, are set forth with particularity in the appended claims. The present invention, both as to its organization and manner of operation, together with further objects and advantages, may best be understood by reference to the following description, taken in connection with the accompanying drawings.

Figure 1 shows a perspective view of an apparatus according to the invention which includes a stationary magnet placed close to a mobile container partially filled with a test medium containing magnetic particles;

Figure 2 shows a perspective view of a different apparatus according to the invention which includes a mobile magnet placed close to a stationary container partially filled with a test medium containing magnetic particles;

Figure 3 shows a perspective view of an apparatus according to the invention which includes a row of mobile magnets placed close to corresponding stationary containers and rotationally displaced by a common mechanism;

Figure 4 shows a perspective view of another apparatus according to the invention which includes a row of stationary magnets placed close to corresponding rotatable containers which are rotated by a common mechanism;

Figures 5a, 5b, 5c, 5d, and 5f schematically illustrate various steps of a method according to the invention for magnetic mixing and magnetic separation of a target substance employing magnetic particles with the help of a magnetic mixing and separation apparatus according Figure 2;

Figure 6 schematically shows a perspective view of a magnetic field gradient "cavity" in a test medium according to an embodiment of the invention which includes one permanent magnet placed close to the container;

Figure 7 schematically shows a perspective view of a magnetic field gradient "cavity" in a test medium according to an embodiment of the invention which includes two magnets placed at the opposite sides of the container;

Figure 8 schematically shows a perspective view of multiple magnetic field gradient "cavities" in a test medium according to an embodiment of the invention which includes a vertical array of six permanent magnets placed close to the container;

Figure 9 schematically shows a perspective view of multiple magnetic field gradient "cavities" in a test medium according to an embodiment of the invention which includes two vertical arrays of permanent magnets placed at the opposite sides of the container;

Figure 10a schematically shows a perspective top view of an apparatus according to the invention which includes two electromagnets placed at opposite sides of the container;

Figure 10b schematically shows a perspective top view of an apparatus according to the invention which includes a ring of electromagnets surrounding the container; and

Figures 11a and 11b depict magnetic field lines in the container generated by two magnets placed on opposite sides of the container.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description is provided to enable any person skilled in the art to make and use the invention and sets forth the best modes contemplated by the inventor of carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the generic principles of the present invention have been defined herein specifically to provide an apparatus and method for mixing samples containing magnetic particles by imparting movement to the particles by means of a magnetic field.

The subject invention provides improved apparatus and methods for affinity-based separation of target substances from a test liquid medium (test medium) by magnetic particles. The invention includes a novel mixing system wherein the magnetic particles are mixed within a relatively motionless test liquid by magnetic means disposed external to the container holding the test liquid. The invention also provides an apparatus in which both the processes of mixing and separation are carried out by a common magnetic means disposed in a single apparatus, thereby making it simpler and more practical to use.

The present invention permits rapid, efficient, and clean separation of target substance from test media and is particularly useful in the affinity magnetic separations of organic, biochemical, or cellular components of interest from, for example, assay reaction mixtures, cell cultures, body fluids and the like.

The apparatus of the invention comprises at least one container for holding a test medium, external magnetic means to generate a magnetic field gradient within the test medium, and means for creating a magnetically-induced movement of the magnetic particles within the test medium. The container is preferably of cylindrical configuration, made of a nonmagnetic material such

as glass or plastic, and provides a chamber for performing the desired mixing and separation. Preferably, the container has at least one opening for receiving the test medium containing the magnetic particles.

The magnetic means may comprise one or more permanent or electromagnets disposed externally to the container for generating magnetic field gradients within the test medium. In a preferred embodiment, the magnetic means is a permanent magnet of a rare earth alloy and is disposed relative to the container so as to define a magnetic field gradient "cavity" in a desired cross-section of the test medium. The term "cavity" is employed because the magnetic field strength gradients act to confine or concentrate the magnetic particles much as if they were enclosed within a cavity.

The magnetic field strength in the cavity is stronger at a part of the internal surface of the container closer to the magnet (locus of magnetic force) than it is elsewhere in the cavity and becomes negligible outside the cavity. As a result, magnetic particles near this locus are subject to considerably greater magnetic force than those farther from it. In certain preferred embodiments, two magnets may be located on the opposite sides of the container, preferably with similar magnetic poles facing each other, to distort the magnetic flux lines and generate two magnetic field gradients and two loci of magnetic force forming in one cavity. Such an arrangement is particularly useful for agitating magnetic particles, as described later on. In a particularly advantageous arrangement, an assembly comprising a vertical array of magnets may be positioned exterior to the container to create multiple magnetic field gradient cavities within a desired cross-section of the test medium.

The present invention provides two methods for agitating and mixing the magnetic particles within the test medium while maintaining the test medium relatively motionless:

(1) moving the magnetic particles through the test medium by rotating the container against a stationary magnet defining a stationary magnetic field gradient cavity, thus inducing an angular movement in the magnetic particles relative to

the essentially motionless test medium caused by change in angular position between the container and the magnetic means; and

(2) moving the magnetic particles within the test medium by rotating the magnet about the stationary container defining a moving magnetic field gradient cavity, and inducing an angular movement of the particles relative to the essentially motionless test medium caused by change in angular position between the container and the magnetic means.

The magnetic field gradient cavity in the test medium tends to confine the magnetic particles and, as the relative angular position between the container and magnet is displaced, induces an angular movement in the magnetic particles, moving them into contact with a large volume of the test medium and enhancing contact with the target substance. Agitation of magnetic particles is significantly improved in a moving cavity of magnetic field gradient wherein the magnetic flux lines are distorted as in the case of two permanent magnets on the opposite sides of the container with similar magnetic poles facing each other shown in Figure 11a.

The magnetic field lines thus generated by the two magnets are mutually repulsive and the cavity is characterized by having two large magnetic fields with corresponding loci of high magnetic attractions and a very small region in the center (neutral zone) where there is virtually no magnetic field. Since this neutral zone is very small, the random motion of magnetic particles caused by Brownian, gravitational, thermal and the like will tend to push most of the magnetic particles into either of the two magnetic fields in the cavity. In a dynamic situation where the relative angular position between the magnets and the container is continuously changing, opposing magnetic flux lines causes the magnetic particles to disperse and mix more efficiently than in the case of a single magnet. However, when two magnets are of opposite poles, as shown in Figure 11b, the magnetic field lines are mutually attractive and the cavity is characterized by having two relatively small magnetic fields with corresponding loci of high magnetic attractions and a large region in the center (neutral zone)

where there is virtually no magnetic field. Although such an arrangement may be of use in certain situations, a large neutral zone and mutually attractive magnetic flux lines are generally less advantageous.

Unlike the methods of mixing disclosed in the prior art, the mixing system of the present invention agitates the magnetic particles in the test medium without significant liquid turbulence; i.e., the test medium remains relatively motionless. Such mixing provides a high rate of contact between the affinity surface of the magnetic particles and the target substance to enhance the affinity bonding, while maintaining the hydrodynamic shear force at the contacting surface at a value less than the affinity bond strength or too low to effect denaturation or other damage. The mixing mechanism employed in the practice of this invention is particularly useful for micrometer-sized magnetic particles and permits a level of operating efficiency which has not been achievable heretofore.

The purity and yield of the target substance obtained by a particular affinity magnetic separation is largely determined by the mixing process employed to promote the affinity binding reaction between a target substance and the surface of the magnetic particles. The binding reactions require a close contact between the affinity surface and the target substance. The rate of the reaction largely depends on the collision frequency between the two entities and the rate of surface renewal of the magnetic particles. The surface renewal is the process of removing the thin layer of media at the affinity surface and exchanging it with fresh media from the bulk. The hydrodynamic shear force at the affinity surface, therefore, must be carefully balanced so that it is sufficient to remove the thin layer of media without disrupting affinity bonds. This has been difficult to achieve by past mixing methods based on agitating the test medium. However, according to this invention, a high collision frequency and a substantially balanced shear force can be achieved by magnetically inducing a controlled movement of the magnetic particles in an essentially motionless test medium.

As the relative angular position between of the magnet and the container change, the point of maximum field strength at the internal surface of the container recedes continuously and induces an angular movement in the

magnetic particles, while the test medium remains relatively stationary with respect to the internal surface of the container. Such an angular movement of the magnetic particles ensures a very effective agitation of the magnetic particles in the test medium by providing optimal exposure of the particles' affinity surface areas to the target substance. At moderate speeds of rotation preferably between about 10 to about 100 revolutions per minute, the test liquid within the container can be considered to remain stationary. Since the hydrodynamic shear force acting at the surface of magnetic particles is essentially governed by the velocity of the magnetic particle movement in the test medium, it may be controlled by adjusting the speed of angular movement.

The separation of magnetic particles from the test medium in accordance with the invention is effected by stopping the rotation of either the magnet or the container as described earlier to terminate the agitation of the magnetic particles. In the stationary position of either the container or the magnet, the magnetic particles within the cavity of magnetic field gradient in the test medium are attracted and immobilized at the inside wall of the container nearest to the magnet. By accomplishing both the mixing and separation operations by means of a single source of magnetic field, the present invention greatly simplifies the entire process and makes the apparatus mechanically less complicated and less costly.

Magnetic mixing and separation according to the present invention have particular utility in various laboratory and clinical procedures involving biospecific affinity binding reactions for separations. In such procedures, magnetic particles are used which have their surface coated with one member of a specific affinity binding pair, i.e. ligand or receptor, capable of specifically binding a substance of interest in the test medium.

Such biospecific affinity binding reactions may be employed for the determination or isolation of a wide range of target substances in biological samples. Examples of target substances are, cells, cell components, cell subpopulations (both eukaryotic and prokaryotic), bacteria, viruses, parasites, antigens, specific antibodies, nucleic acid sequences and the like. Thus, the apparatus and method of the invention may be used to carry out immunospecific cell separations for the analysis or isolation of cells including, by way of

example: tumor cells from bone marrow; T-lymphocytes from peripheral blood or bone marrow; lymphocyte subsets, such as CD2, CD4, CD8, and CD19 from peripheral blood, monocytes; granulocytes and other cell types. The removal or depletion of various cell types may be carried out in a similar manner. The present invention may be also be used in the separation or analysis of various bacteria or parasites from food products, culture media, body fluids and the like. Similarly, the present apparatus and method may be used in: bioassays including immunoassays and nucleic acid probe assays; isolation and detection of DNA and mRNA directly from crude cell lysate; and isolation and detection of proteins.

The type of magnetic particles useful for the practice of the invention are noncolloidal and are paramagnetic; that is, they are magnetizable but do not retain any magnetism after the magnetic field is removed. Such magnetic particles are typically of polymeric material containing a small amount of ferro-magnetic substance such as iron-based oxides, e.g., magnetite, transition metals, or rare earth element, which causes them to be captured by a magnetic field. The paramagnetic particles useful for practicing the invention should provide for an adequate binding surface capacity for the adsorption or covalent coupling of one member of a specific affinity binding pair, i.e. ligand or receptor, and are typically of diameters between 0.1 to 10 μm. Suitable paramagnetic particles are commercially available from Dynal Inc. of Lake Success, NY; PerSeptive Diagnostics, Inc., of Cambridge, MA; and Cortex Biochem Inc., of San Leandro, CA. The preferred particles, being those sold under the identification numbers M-280 and M-450 by Dynal Inc., are of uniform sizes of 2.8 and 4.5 μm in diameter, respectively, and contain magnetizable material evenly dispersed throughout. These beads are coated with a thin shell of polystyrene which provides a defined surface for the immobilization of various ligands or receptors. Such immobilization may be carried out by any well-known techniques; techniques employing either physical adsorption or covalent coupling chemistry are preferred.

The magnetic field gradients may be generated by one or more permanent magnet(s) or electromagnet(s). Permanent magnets are preferred for most mixing and separation devices such as those employed in laboratory-scale

operations and for automated devices employed in clinical diagnostics. However, larger scale devices or automated devices such as those employed in pharmaceutical or industrial production can be more advantageously produced using electromagnets, since the field gradients can be more easily altered under automatic control to effect various processing steps.

Permanent magnets useful for practicing the invention should have a surface field strength sufficient to attract a majority of magnetic particles. Permanent magnets of rare earth alloys having a surface field strength in the range of one to several tens of kG (kiloGauss) are preferred. Permanent magnets made from Neodymium-Iron-Boron or Samarium-Cobalt magnets and characterized by BH_{max} (maximum energy product) in the range of 10 to 35 mGOe (megaGauss Oersted) are particularly preferred. Such magnets may be obtained from International Magnaproducts Inc., of Valparaiso, IN, and many other commercial sources. Preferably the permanent magnets have a rectangular cross-section and may be glued or fixed by mechanical means to a nonmagnetic holding support to form a permanent magnet assembly. The assembly may include a ferromagnetic harness to house the magnet or magnets and to focus the magnetic field. The magnets are preferably oriented with their magnetic field axis perpendicular to the vertical axis of the container. Alternate cross-sectional shapes and orientations of magnets are also envisioned to be well within the scope of the invention.

Generally the permanent magnet assembly is placed in close proximity to the container without the magnet extending to the bottom of the container. The preferred distance between each magnet assembly and the container shown in the apparatus of Figures 1 through 6 is generally about 5 mm to about 20 mm. The field strength of the magnet or magnets should be great enough and the distance between the magnet and the container short enough to generate an effective cavity of magnetic field gradient in the test medium. In certain situations involving the processing of a plurality of containers, it may be advantageous to place the permanent magnet assembly between containers or between rows of containers so that one single permanent magnet assembly can be used to generate a magnetic field cavity in the two containers in its vicinity.

Figure 1 illustrates an apparatus for mixing and separating magnetic particles according to the present invention which includes a magnet 1 fixed to a solid support 2 placed in close proximity to a cylindrical container 3 without extending to the container's bottom end. In the illustrated embodiment, the container 3 used to hold a test medium 8 is a test tube and magnetic particles 9 are shown as small dots. If the magnet 1 is a permanent magnet, it preferably comprises a rare earth composite type such as Neodymium-Iron-Boron or Samarium-Cobalt and has a surface field strength sufficient to attract the magnetic particles, preferably a BH_{max} of over 20 mGOe. If an electromagnet is employed for the magnet 1, it should have a comparable field strength.

The container 3 containing the test medium 8 and the magnetic particles 9 is removably placed in a vertical position in a holder 5 fixed to a rotating shaft 4 attached to a variable speed electric motor 6. The holder 5 has vertical slits 7 which are elastic and firmly grip the container 3. Switching on the electric motor 6 rotates the container 3, thereby causing the relative angular position of the container 3 to the magnet 1 to be continuously altered, inducing the magnetic particles 9 to move within the cavity of the magnetic field gradient defined within the test medium 8. The container 3 is rotated at a moderate speed, preferably between 10 and 100 revolutions per minute, to ensure the agitation of the magnetic particles 9, while the test medium 8 inside remains relatively stationary. Switching off the electric motor 6 stops the rotation of the container 3, and the magnetically-induced agitation of the magnetic particles 9 ceases. At this time the magnetic particles 9 are attracted and immobilized at the inside wall of the container 3 closest to the magnet 1. The congregation of the magnetic particles 9 on the vertical side of the container 3 facilitates removal of the test medium 8.

Figure 2 illustrates another apparatus for mixing and separating magnetic particles according to the present invention which includes a test tube 23 fixed removably in a vertical position at its top end through an opening in a test tube holder 25 with a magnet 21 placed in close proximity to the test tube 23 without extending to its bottom end. Again, the magnet 21 may be either an electromagnet or a permanent magnet. If permanent magnet, the magnet 21

is preferably comprised of a rare earth composite such as Neodymium-Iron-Boron or Samarium-Cobalt and has a surface field strength sufficient to attract the magnetic particles 9, preferably a BH_{max} value of over 20 mGOe. The magnet 21 may comprise one or more magnets of suitable dimensions and geometries so as to define a magnetic field cavity which accommodates a desired cross-section of the test medium 28 in the test tube 23.

The magnet 21 is fixed on a disc 22 which is mounted on a rotating shaft 24 attached to a variable speed electric motor 26. Again, switching on the electric motor 26 rotates the magnet 21 orbitally around the vertical axis of the stationary test tube 23, thereby creating an angularly moving magnetic field gradient within the test tube 23. During rotation, the test tube 23 remain motionless while the magnetic field cavity rotates continuously through the stationary test medium 28. The angularly-moving magnetic field induces the magnetic particles 29 to move within the cavity of the magnetic field gradient in the test medium 28. The magnet 21 is rotated at a moderate speed, preferably between 10 to 100 revolution per minute, to move the magnetic particles 29 through the essentially motionless test medium 28. When the electric motor 6 is switched off, the rotation of the magnet 21 ceases, thereby stopping the magnetically-induced agitation. The magnetic particles 28 in the now stationary magnetic field are attracted to and immobilized on the inside wall of the test tube 23 closest to the magnet 21. Congregation of the magnetic particles on the vertical side of the test tube 23 facilitates the removal of the test medium 28 by aspiration or other means.

Figure 3 illustrates an embodiment of an apparatus in accordance with the present invention for processing a plurality of test media simultaneously and is a variant of the apparatus described in Figure 2. The apparatus according to Figure 3 comprises a row of identical test tubes 33, fixed in vertical positions by their the top ends passing through corresponding openings in a fixed horizontal support-plate 32, and a corresponding row of magnets 31 aligned in close proximity to the test tubes 33 without extending to their bottom ends. If permanent magnets are used, they are preferably of rare earth types

as described in relation to Figure 2, and are selected to be of suitable dimensions and geometries to define a magnetic field cavity which accommodates a desired cross-section of the test medium 29 in each test tube 33.

The row of magnets 31 is mounted on a mobile support plate 35 fixed at its extremities by two shafts 34a and 34b which are eccentrically attached to pulleys 38a and 38b which are, in turn, connected by a drive belt 39. The pulley 38a is attached to a variable speed electric motor 36 so that switching on the motor 36 rotates the pulleys 38a and 38b, thereby imparting an eccentric rotation to support plate 35. This motion causes each magnet 31 to orbit around the vertical axes of its corresponding stationary test tube 33, thereby creating a separate moving magnetic field gradient within the motionless test media 28 of each test tube 33. The simultaneous movement of multiple magnetic fields induces the magnetic particles in each test tube 33 to move within their individual cavity of the magnetic field gradient. Switching off the electric motor 36 stops the rotation of the magnets 31 and the magnetically-induced agitation ceases. The magnetic particles 29 in the now stationary magnetic fields are attracted to and immobilized on the inside walls of each test tube 33. The separation of magnetic particles on the vertical side of the test tubes 33 facilitates the removal of supernatant liquid media by aspiration or other methods.

Figure 4 illustrates another embodiment of an apparatus in accordance with the present invention for processing a plurality of test liquid media simultaneously, and is a variant of the apparatus described in Figure 1. The apparatus according to Figure 4 comprises a row of magnets 41 fixed on a vertical plate 42c which is part of a test tube rack 42. The magnets 41 are aligned in close proximity to the row of test tubes 43 without extending to their bottom ends. If desired, the magnets 41 may be placed between alternating test tubes 43 so that only one magnet is needed for two adjacent test tubes 43—yielding a simpler and more economical apparatus. If permanent magnets are employed, they are preferably of rare earth types described in Figure 1 and are of suitable dimensions and geometries so as to define a magnetic field cavity which accommodates a desired cross-section of the test medium 8 in each test tube 43.

The test tubes 43 are removably disposed in vertical positions with their bottom ends resting in a row of shallow grooves on a bottom plate 42a and with a portion of their top ends passing through corresponding openings in an upper plate 42b of the test tube rack 42. The diameter of the openings in the upper plate 42b is slightly larger than the diameter of the test tubes 43 so that they can be readily inserted and freely rotated. The plates 42a and 42b are spaced apart so as to hold the test tubes 43 in a stable vertical orientation.

A drive belt 49 is mounted on two pulleys 48b and 48c attached to a variable speed motor 46, and guided by two parallel rows of guidance rollers 47 mounted on the top plate 42b. The guidance rollers 47 are positioned between the row of openings so as to slightly pinch the drive belt 49 so that it grips the upper ends of the test tubes 43. Switching on the motor 46 moves the drive belt 49 and the linear sliding friction of belt 49 simultaneously rotates all test tubes 43 around their vertical axes.

As test tubes 43 rotate, the relative angular position of each test tube 43 and its corresponding magnet 41 is continuously altered, which induces the magnetic particles 9 to move within the cavity of the magnetic field gradient. The test tubes 43 are rotated at a moderate speed, preferably between 10 and 100 revolution per minute, to ensure the agitation of the magnetic particles 9, while the test media 8 inside remains relatively stationary. Switching off the electric motor 46 stops the rotation of test tubes 43, and the magnetically-induced agitation ceases. The magnetic particles 9 in each test tube 43 are attracted to and immobilized at the inside wall closest to the magnet 41. The aggregation of the magnetic particles 9 on the vertical side of the test tubes 43 facilitates removal of the test medium 8 by aspiration or similar methods.

Figures 5a through 5f illustrate typical steps in a method in accordance with the preferred embodiment using affinity reactive magnetic particles for the purpose of bioassays or the isolation cellular of molecular species from a sample solution or suspension of biological fluids. Figure 5a shows an apparatus according to Figure 2, in which a suspension of magnetic particles 58 in a sample solution is dispensed with a pipette 59 into test tube 23. The apparatus is turned on and the magnetic particles 58 are mixed by rotating

the magnet 21 around the test tube 23. Figure 5b shows the same apparatus when mixing is completed and the rotation of the magnet 21 has stopped. The magnetic particles 58 are immobilized against the inner wall of test tube 23 closest to the stationary magnet 21. Figure 5c shows the same apparatus during a washing step in which the collected magnetic particles 58 are washed by introducing an outlet tube 59a to aspirate the supernatant test medium and introducing an inlet tube 59b to add a suitable wash solution into the test tube 23. The magnetic particles 58 are then mixed in the wash solution and separated from it to allow removal of the wash, as described earlier. The washing step may be repeated as many times as required.

Figure 5d shows the same apparatus while stopped for the addition of one or more reagent solutions by pipette 59 for effecting a desired analytical reaction for a bioassay or a chemical displacement reaction to elute the target substance from the magnetic particles 58. Figure 5e shows the same apparatus again turned on for dispersing and mixing the magnetic particles 58 for carrying out said desired reaction. Figure 5f shows the same apparatus stopped to separate magnetic particles 58 from the reaction medium. In the case of bioassays, the supernatant liquid may be measured by any desired measurement method, either directly in test tube 23 or by transferring it elsewhere. For the purpose of isolating a cellular or molecular species, the supernatant may be transferred to a suitable container for subsequent treatment as desired.

Various configurations of magnet assemblies and their position with respect to the container will now be described with reference to Figures 6 through 9. Figure 6 shows a perspective view of an embodiment of the magnet assembly 61 according to the invention wherein a rectangular permanent magnet 62 is fixed on a nonmagnetic base 63 and placed in proximity to a container 64 to generates a cavity of magnetic field gradient 65 in a cross-section of a test medium 66. The usable magnetic field remains mostly confined within this cavity, i.e., there is negligible field strength outside the cavity.

Figure 7 shows two magnet assemblies, 71a, 71b, each comprised of two rectangular permanent magnets 72a and 72b fixed on two nonmagnetic bases 73a and 73b, respectively. The two magnet assemblies 71a, 71b are

located on the opposite sides of a container 74 with similar magnetic poles facing each other to distort the magnetic flux lines and generate a cavity of magnetic field gradient 75 in the test medium 76 and two loci of magnetic force in the cavity 75 (see Figure 11a) as explained above. Such an arrangement has been found to be particularly effective for mixing magnetic particles.

Figure 8 exemplifies an embodiment of a magnet assembly 81 designed to generate multiple cavities of magnetic field gradient in a container 84 and illustrates an array of six evenly-spaced rectangular permanent magnets 82a to 82f fixed on a nonmagnetic support frame 83. The magnet assembly 81 is placed close to the container 84 to generates six cavities of magnetic field gradient 85a to 85f in a test medium 86.

Figure 9 shows two such magnet assemblies 91a and 91b, each comprising of an array of six evenly-spaced rectangular permanent magnets 92a to 92f fixed on two nonmagnetic support frames 93a and 93b, respectively. The two magnet assemblies 91a and 91b are located on the opposite sides of a container 94 with like magnetic poles facing each other. Six cavities of magnetic field gradient 95a to 95f thus generated in a test medium 96 have distorted magnetic flux lines of two operative magnetic fields in each cavity.

The various configurations of magnet assemblies and position as described above may be advantageously employed in the apparatus of the invention depicted in Figures 1 to 4 of the present invention.

As mentioned above, permanent magnets and electromagnets are interchangeable in most configurations of the present invention. However, it is apparent to one skilled in the art that those configuration that require movement of the magnet are more easily realized with permanent magnets because electromagnets require commutators or other arrangements to conduct electricity to the moving magnets. However, there are certain unique configurations in which electromagnets are greatly preferred. Figure 10a shows two electromagnet coils 101a and 101b mounted on a support frame 104 and placed at about 180 degrees at the exterior of a container 102 with the test medium and magnetic particles 103. Figure 10b shows a cross-section of a

single container 102 with the test medium and magnetic particles 103 surrounded by a ring of individual electromagnet coils 101a to 101r mounted on a support frame 104.

Here neither the container 102 nor the electromagnets 101 actually move. Instead, angular movement is induced in magnetic particles suspended within the test medium 103 inside the container 102 by sequentially energizing the electromagnets. This sequential energization may be "binary" (i.e., on and off) or "analog," in which a first electromagnet is gradually fully energized, and then has its power reduced, while the next electromagnet is gradually energized, and so on. It will be apparent that rate of motion of the magnetic particles 103 can be modulated by the rate of change and the degree of overlap between the sequential electromagnets.

The exact number of sequential electromagnets employed will depend on the size of the container 102 and other parameters. Figure 10a shows that this configuration reduces to a configuration not unlike that of Figure 7, but with two opposed electromagnets rather than two permanent magnets. The angular movement from one magnet to the other is actually 180 degrees so that the magnetic particles in the test medium 103 actually move in relatively straight lines back and forth across the container 102. In this situation in particular, more variety can be added to the paths of the magnetic particles by modulating the polarity, as well as the power level of the electric current, thereby altering the direction of the magnetic poles with alterations of the magnetic field corresponding to those shown in Figures 11a and 11b.

It has been found that a configuration employing four electromagnets equally spaced (i.e., 90 degrees apart) around a container can produce very acceptable agitation of magnetic particles through a judicious use of sequential activation of the electromagnets and through polarity reversals, as discussed above.

The container defining the mixing and separation chamber includes at least one opening for the addition and removal of a test medium. The container is preferably of substantially cylindrical form and made from a magnetically permeable material such as plastic or glass. Additionally, the inside surface of the chamber may be biocompatible and, if desired, the

chamber may be sterilized for aseptic processing of the test media. The volume of the container is not critical as long as an adequate magnetic field gradient can be provided to accommodate the chamber and, particularly, a desired cross-section of the test medium inside.

As shown in Figures 1 through 9, the container used to hold the test medium may be a test tube. The volumetric capacity of the test tube is preferably between about 1 ml to about 300 ml, and the size and geometry of the magnet is adjusted to generate an adequate magnetic field gradient within the test medium inside a particular size of test tube.

Although embodiments of the present invention particularly suited for use in the research laboratory preferably employ readily removable and replaceable containers such as test tubes, diagnostic and other devices employing the teachings of the present invention might employ permanent flow cells or other nonremovable chambers for mixing and separation.

According to the present invention, the affinity reactive magnetic particles are admixed with the test medium in a container by effecting a relative angular movement of the magnetic particles in the test medium, which remains essentially motionless. A relative angular movement may be induced in the magnetic particles by either rotating a magnetic field around a stationary container or rotating the container relative to an immobile magnetic field. The magnet creating the field is disposed outside the container and defines a cavity of magnetic field gradient within the test medium. The present invention also contemplates the use of doughnut-shaped containers so that while the magnetic source is "outside" of the container it is actually "within" the container in the sense that it occupies the hole of the doughnut.

Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope and spirit of the invention. Therefore, it is to be understood that, within the scope of the appended claims, the invention may be practiced other than as specifically described herein.

CLAIMS

What Is Claimed Is:

1. An apparatus for mixing and separating magnetic particles in a liquid test medium, the apparatus comprising:

a magnetically permeable container for receiving said test medium and providing a chamber for magnetic mixing and separation;

a holder for holding said container;

magnetic means disposed outside of said container held in said holder for generating a magnetic field gradient within said container, the magnetic field being stronger at a point on an inner surface of a lateral wall of said container closest to the magnetic means, thereby defining a magnetic field cavity within said test medium;

moving means for continuously changing the relative angular position between said container and the magnetic means to induce a movement of said magnetic particles in said test medium; and

separation means for separating said magnetic particles from said test medium, the separation means operating to stop the moving means, thereby immobilizing said magnetic particles on the inner surface of the lateral wall of said container nearest to the magnetic means.

2. The apparatus of Claim 1, wherein the magnetically permeable container is of a substantially cylindrical configuration.

3. The apparatus according to Claim 1, wherein said magnetic means comprises at least one permanent magnet.

4. The apparatus according to Claim 1, wherein said magnetic means comprises at least one electromagnet.

5. The apparatus according to Claim 1, wherein said magnetic means has a maximum energy product between about 10 and about 30 megaGauss Oersted.

6. The apparatus of Claim 3, wherein the permanent magnet comprises a magnetic alloy containing rare earth elements.

7. The apparatus according to Claim 1, wherein said magnetic means comprises a plurality of magnets disposed vertically to define a vertically-arranged plurality of magnetic field cavities, and wherein the separation means causes magnetic particles to become immobilized within each of said cavities.

8. The apparatus according to Claim 1, wherein the moving means orbitally moves said magnetic means around an outer surface of the lateral wall of said container, said container remaining stationary.

9. The apparatus of Claim 8, wherein said magnetic means is mounted on a mobile base member operationally connected to a motor with said container suspended above said member so that operation of the motor rotates said member, causing said magnetic means to orbit closely about the outer surface of the lateral wall of said container.

10. The apparatus of Claim 1, wherein the magnetic means comprises two magnets disposes on opposite sides of said container with like magnetic poles facing said container.

11. The apparatus according to Claim 1, wherein the moving means rotates said container with respect to said magnetic means, said magnetic means remaining substantially stationary.

12. A method of mixing magnetic particles in a test medium for carrying out an affinity binding reaction between a target substance and said particles so as to maximize contact between an affinity surface of said particles and the target substance, minimize turbulence and shear forces between said particles and said medium, and simplify the separation of said particles from said medium, the method comprising the steps of:

placing said test medium and said magnetic particles into a magnetically permeable container;

mixing said magnetic particles within said test medium to effect contact between said target substance and the affinity surface of said magnetic particles using a magnetic source disposed externally to said container to impart a movement to said particles relative to said test medium, the movement being effected by changing the relative angular position between said container and the magnetic source; and

separating said particles from said test medium by making the magnetic source and said container stationary relative to each other so that said particles concentrate on an interior surface of said container closest to the magnetic source, allowing removal of said test medium without disturbing said concentrated particles.

13. The method according to Claim 12, wherein the mixing and the separating steps are repeated to permit addition and removal of washing and reaction solutions.

14. An apparatus for mixing and separating magnetic particles in a plurality of nonmagnetic test media in which said magnetic particles are suspended, the apparatus comprising:

a plurality of magnetically permeable containers for receiving the plurality of said test media providing chambers for magnetic mixing and separation processes;

a holding means for holding said containers;

a plurality of magnetic means externally aligned with said containers held by the holding means for generating a magnetic field gradient within each container, each magnetic means disposed laterally near an exterior surface of its corresponding container for defining a magnetic field cavity within the test medium of said container;

moving means for changing the relative angular position between each container and its corresponding magnetic means to induce a movement of said magnetic particles in said test medium, thereby ensuring mixing of said particles with said test medium; and

separation means for separating said magnetic particles from said test media by stopping the change in the relative angular positions between each container and its corresponding magnetic means, whereby said magnetic particles are immobilized against an inner lateral surface of each container nearest to its magnetic means, permitting the separation of said test medium from said magnetic particles.

15. The apparatus according to Claim 14, wherein said magnetic means are permanent magnets.

16. The apparatus according to Claim 14, wherein said magnetic means are electromagnets.

17. The apparatus of Claim 15, wherein the permanent magnets comprise a magnetic alloy containing rare earth elements.

18. The apparatus according to Claim 14, wherein the moving means orbitally moves each of said magnetic means around an exterior lateral wall of its corresponding container, said container remaining substantially stationary.

19. The apparatus according to Claim 14, wherein the moving means rotates said containers with respect to said magnetic means, said magnetic means remaining substantially stationary.

20. The apparatus of Claim 14, wherein the magnetic means comprises two magnets disposes on opposite sides of said container with like magnetic poles facing said container.

21. The apparatus according to Claim 14, wherein each magnetic means comprises a plurality of magnets disposed vertically to define a vertically-arranged plurality of magnetic field cavities in the corresponding container, and wherein the separation means causes magnetic particles to become immobilized within each of said cavities.

22. An apparatus for mixing and separating magnetic particles in a liquid test medium, the apparatus comprising:

a magnetically permeable container for receiving said test medium and providing a chamber for magnetic mixing and separation;

a holder for holding said container;

a plurality of electromagnets disposed outside of said container held by the holder for generating a magnetic field gradient within said container;

moving means for changing the relative angular positions of said container and the magnetic field gradient to induce a movement of said magnetic particles in said test medium, said moving means operating to sequentially modulate at least one of a level of electric power energizing each electromagnet and a polarity of electric power energizing each electromagnet; and

separation means for separating said magnetic particles from said test medium, said separation means causing a cessation of modulation of electric power and energizing at least one electromagnet, thereby immobilizing said magnetic particles on an inner surface of a lateral wall of said container nearest to the energized electromagnet.

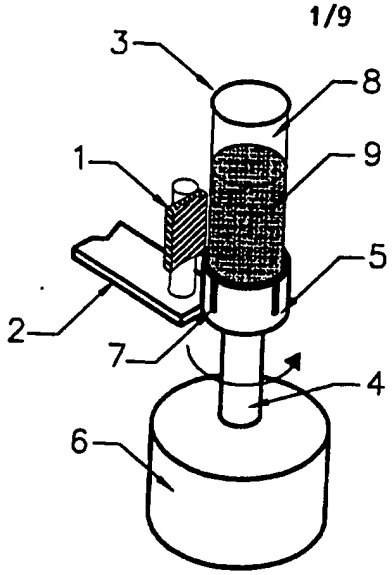

FIG. 1

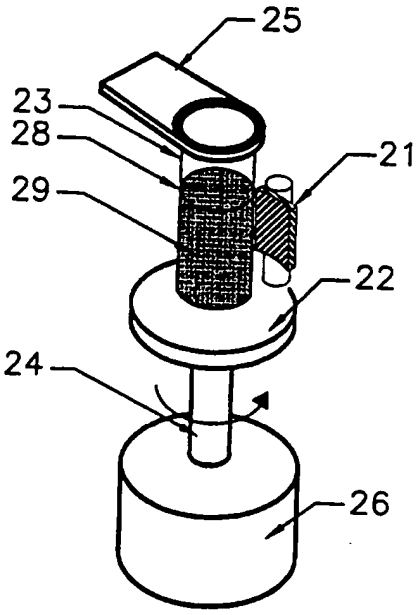

FIG. 2

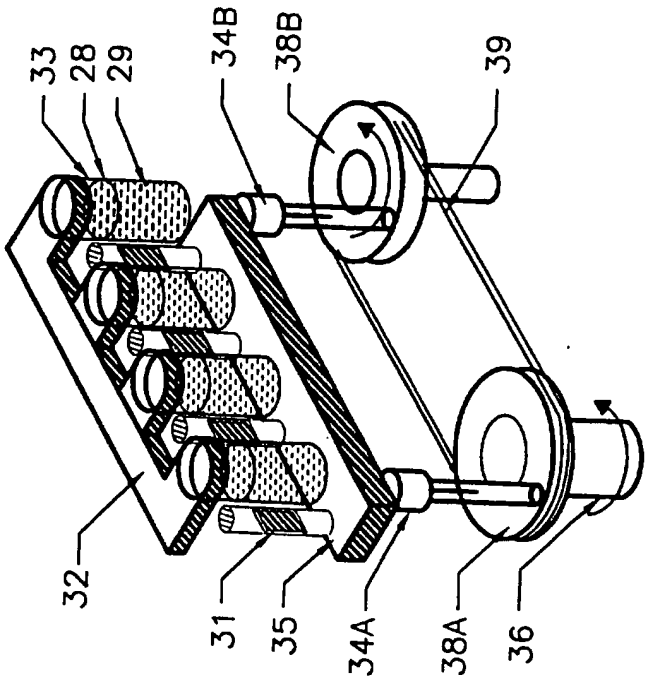

FIG. 3

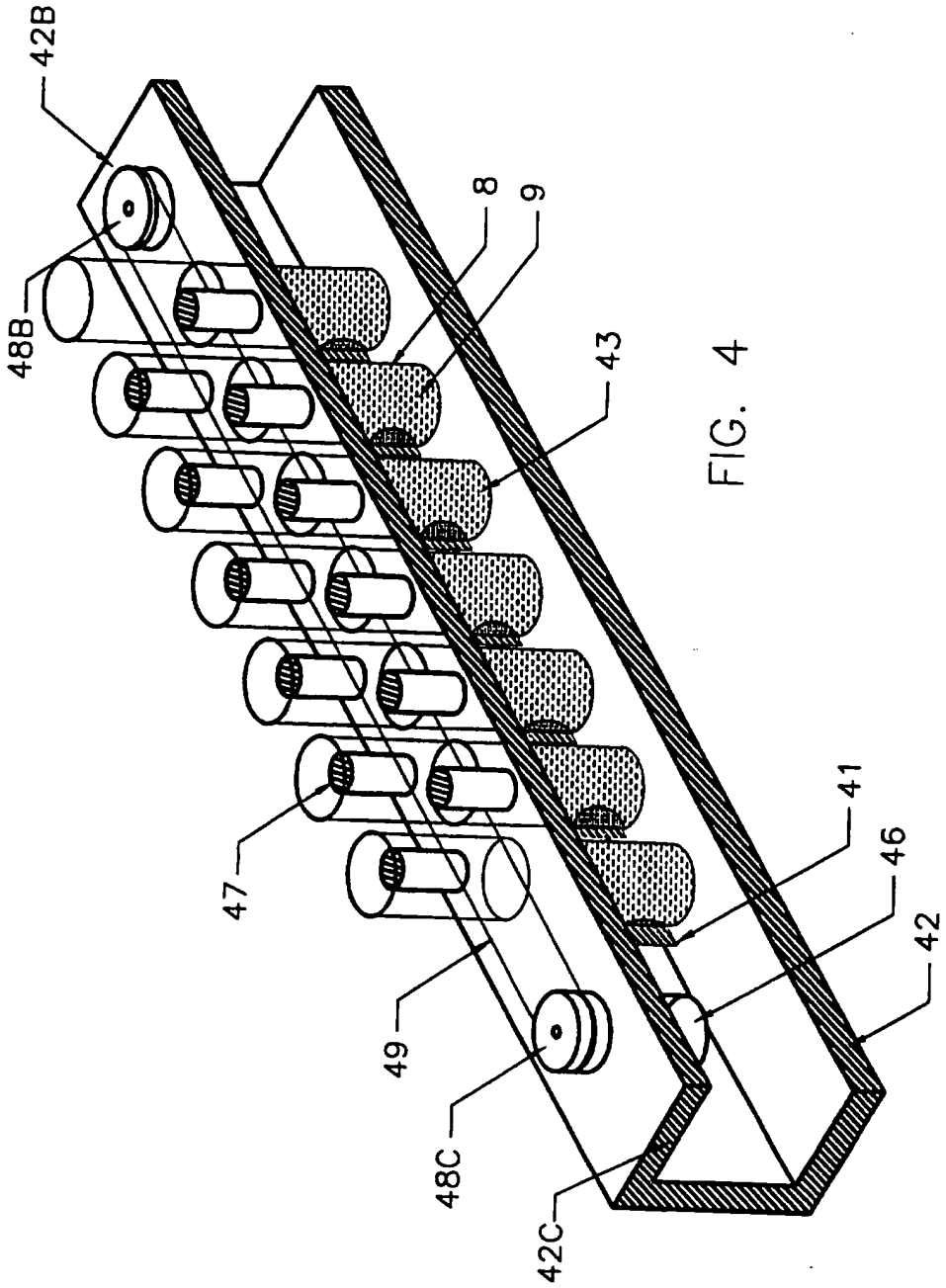

FIG. 4

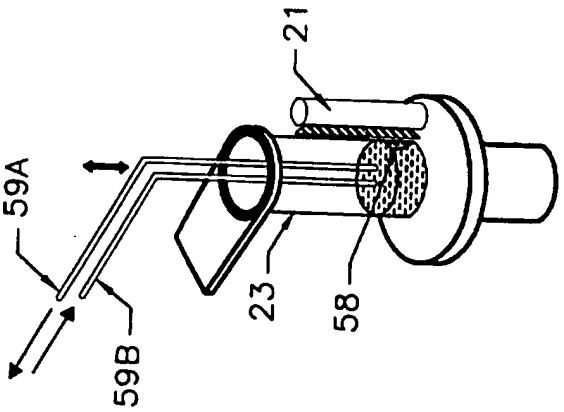

FIG. 5C

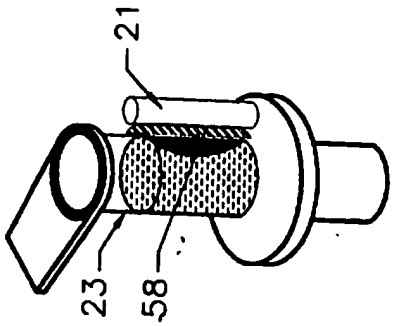

FIG. 5B

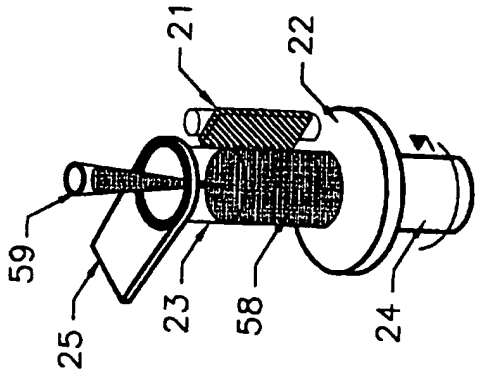

FIG. 5A

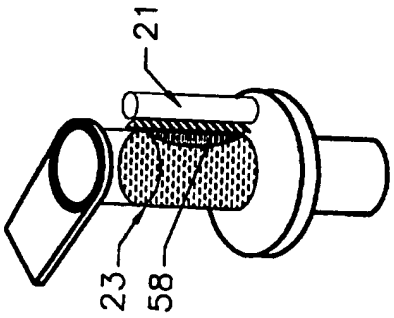

FIG. 5F

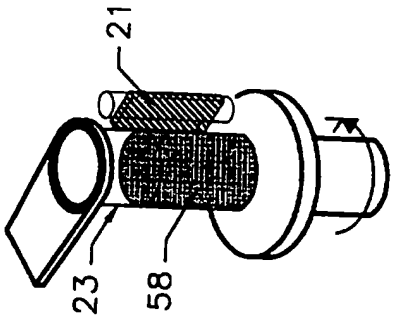

FIG. 5E

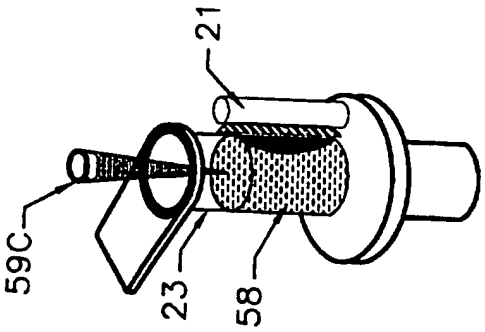

FIG. 5D

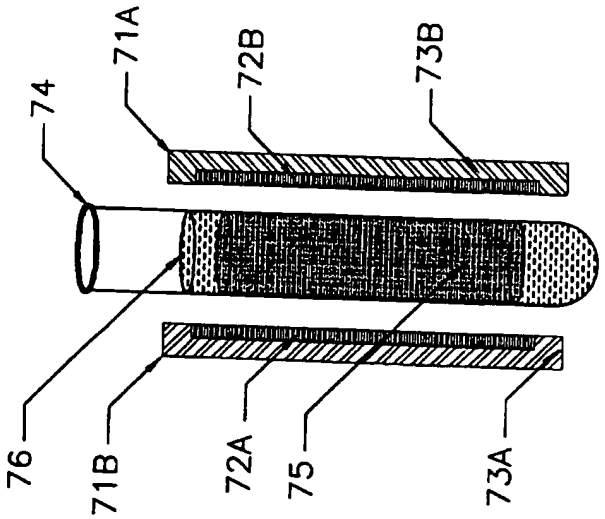

FIG. 7

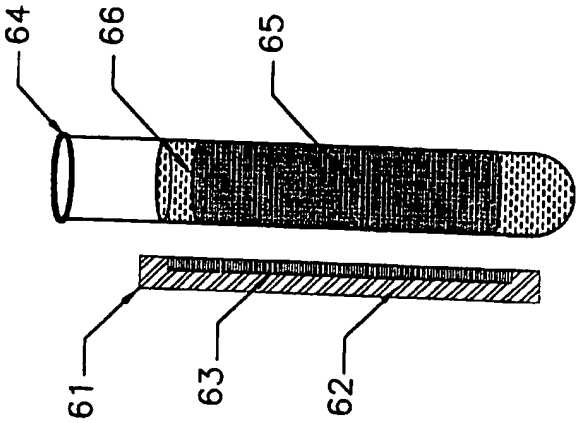

FIG. 6

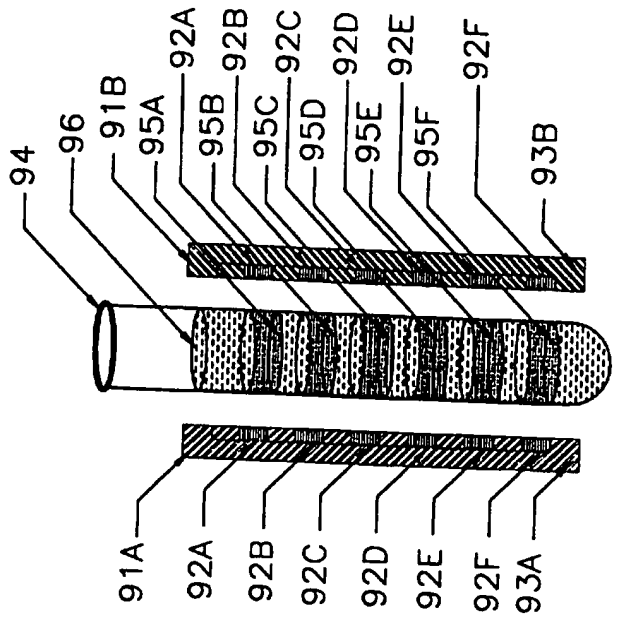
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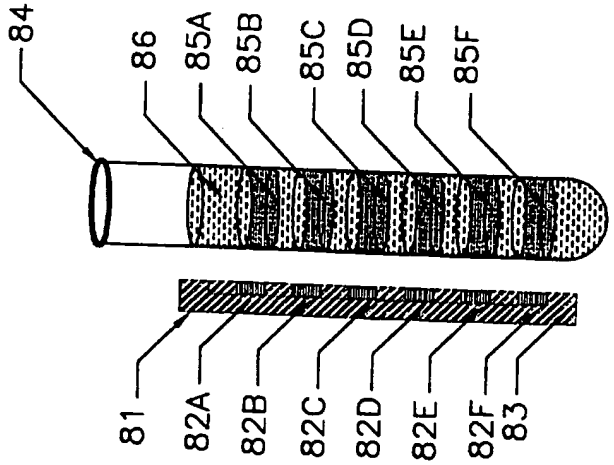
FIG. 9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83

FIG. 8

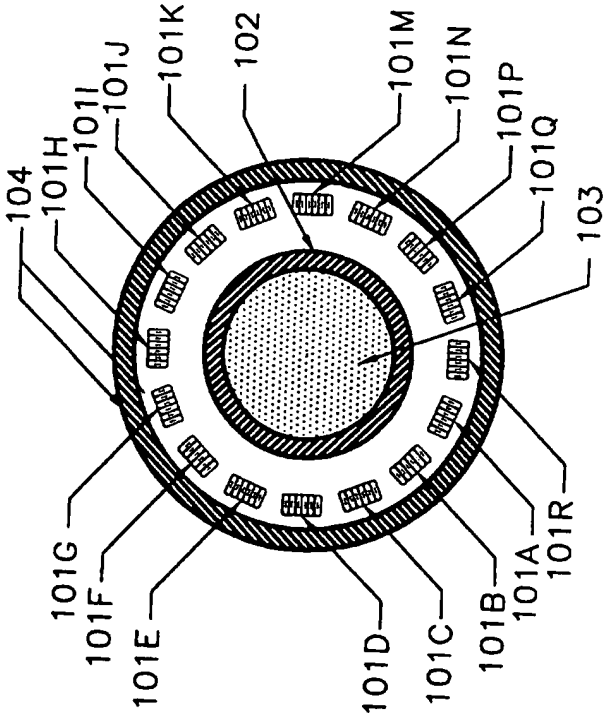

FIG. 10B

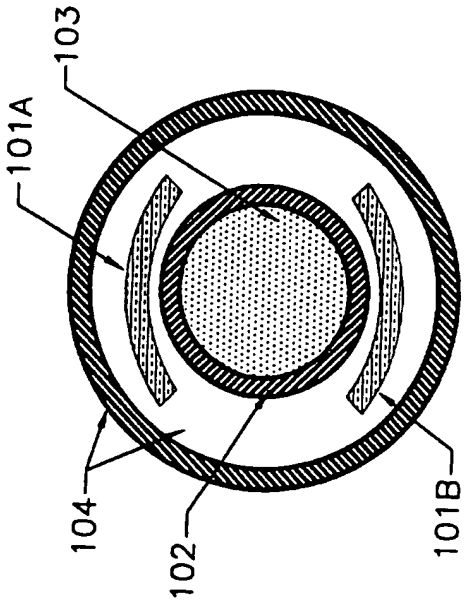

FIG. 10A

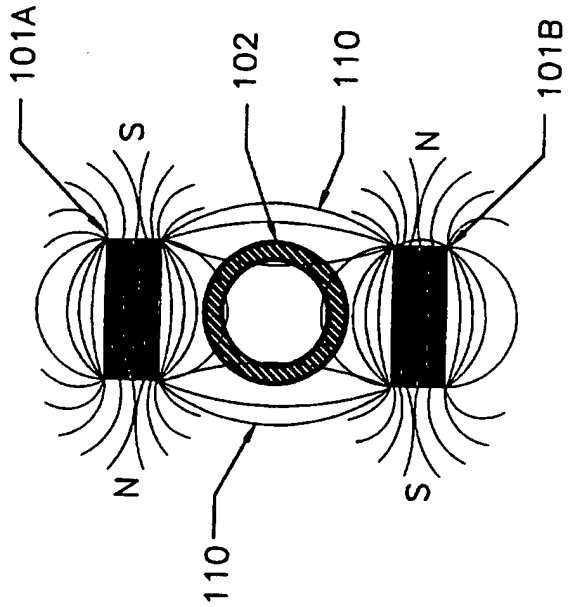

FIG. 11B

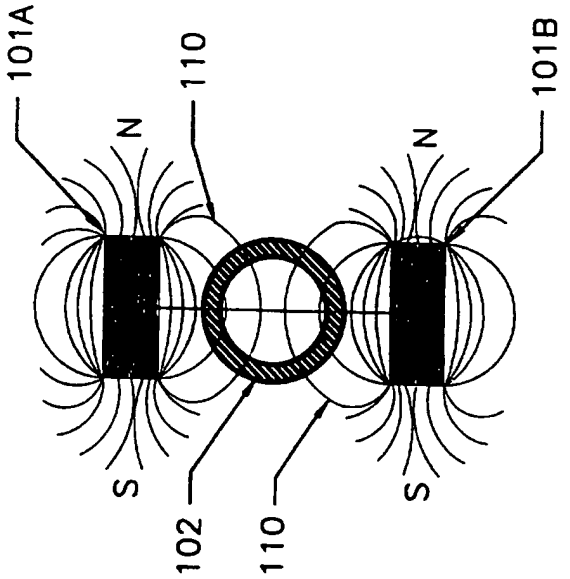

FIG. 11A

INTERNATIONAL SEARCH REPORT

Inte· nal Application No
PCT/US 96/02212

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B03C1/28 B03C1/24 B03C1/01 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B03C G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	SU,A,1 245 343 (UKR GEOL MIN RESOUR) 23 July 1986 see the whole document	1,3,12, 13 4,8
Y A	US,A,5 336 760 (HARDWICK R ALAN ET AL) 9 August 1994 see column 13, line 4 - line 18; claims 1,16; figures 1,2	1,3,12, 13 2,5-7
A	EP,A,0 026 700 (COMMISSARIAT ENERGIE ATOMIQUE) 8 April 1981 see claims 1,2,4,5	1
A	PATENT ABSTRACTS OF JAPAN vol. 007, no. 077 (C-159), 30 March 1983 & JP,A,58 008562 (NIPPON GENSHIRYOKU KENKYUSHO;OTHERS: 03), 18 January 1983, see abstract	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 1996	17.05.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Decanniere, L

Form PCT ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

..nformation on patent family members

Inte nal Application No
PCT/US 96/02212

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
SU-A-1245343	23-07-86	NONE		
US-A-5336760	09-08-94	CA-A-	2067158	15-03-91
		EP-A-	0491858	01-07-92
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